IN THE SPECIFICATION:

Please amend the Specification as follows:

Please replace pages 3-4, 6-18 and 26 with the pages attached hereto. Both a clean version of the amended pages, as well as a marked up version are submitted herewith as per 37 CFR 1.125.

acids in the peptide linker is selected from the group consisting of (Gly, Ser, Asn, Thr and Ala; the peptide linker includes a Gly-Ser element.

In a preferred embodiment, the fusion protein includes a peptide linker and the peptide linker includes a sequence having the formula (Ser-Gly-G1y-G1y-G1y)y (SEQ. ID 1) wherein y is 1, 2,3, 4, 5, 6,7, or 8. Preferably, the peptide linker includes a sequence having the formula $(Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)_3$ (SEQ. ID 1). Preferably, the peptide linker includes a sequence having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly[_3]_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3).

In a preferred embodiment, the fusion protein includes a peptide linker and the peptide linker includes a sequence having the formula (Ser-Ser-Ser-Ser-Gly)y (SEQ. ID 4) wherein y is 1,2, 3, 4, 5,6, 7, or 8. Preferably, the peptide linker includes a sequence having the formula $((Ser\text{-}Ser\text{-}Ser\text{-}Ser\text{-}Gly)_3\text{-}Ser\text{-}Pro)$ (SEQ. ID 4).

In another aspect, the invention features, an EPOa-hSA fusion protein wherein the EPOa includes amino acid residues G1n24, G1n38, G1n83 and A1a126.

In a preferred embodiment the EPOa is G1n24, 01n38, Gln83, A1a126 EPO (i.e., only amino acids 24, 38, 83, and 126 differ from wild type).

In another aspect, the invention features, an EPOa-hSA fusion protein which includes from left to right, an EPOa which includes amino acid residues G1n24, Gln38, G1n83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}GIy\text{-}Gly)[_3]_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the EPOa is G1n24, G1n38, G1n83, A1a126 EPO.

In a preferred embodiment the fusion protein is from left to right, G1n24, G1n38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)[_3]_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In another aspect, the invention features, an EPOa-hSA fusion protein which includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)[_3]_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues G1n24, G1n38, G1n83 and Ala126.

In a preferred embodiment the EPOa is G1n24, G1n38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)[_3]_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, G1n83, A1a126 EPO.

In another aspect, the invention features, an isolated nucleic acid having a nucleotide sequence which encodes an EPOa-hSA fusion protein described herein, e.g., an EPOa-hSA fusion protein wherein at least one amino acid residue is altered such that a site which serves as a site for glycosylation in EPO does not serve as a site for glycosylation in the EPOa, e.g., an EPOa-hSA fusion protein in which at least one amino acid residue of the encoded EPOa-hSA which can serve as a glycosylation site in erythropoietin is altered, e.g., by substitution or deletion, such that it does not serve as a glycosylation site.

In another aspect, the invention features, a nucleic acid which encodes an EPOa-hSA fusion protein wherein the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a nucleic acid which encodes an EPOa-hSA fusion protein which includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In another aspect, the invention features, a nucleic acid which encodes an EPOa-hSA fusion protein which includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, an expression vector or a construct which includes a nucleic acid of the invention.

linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

The invention also includes a cultured cell which includes a nucleic acid which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein. The invention also includes methods of making such cells, e.g., by introducing into the cell, or forming in the cell, a nucleic acid which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein.

In another aspect, the invention features, a method of making an EPOa-hSA fusion protein, e.g., an EPOa-hSA described herein. The method includes providing a transgenic organism which includes a transgene which directs the expression of EPOa-hSA fusion protein; allowing the transgene to be expressed; and, preferably, recovering a transgenically produced EPOa-hSA fusion protein, e.g., from the organism or from a product produced by the organism.

In a preferred embodiment, the transgenic organism is a transgenic animal, e.g., a transgenic mammal, e.g., a transgenic dairy animal, e.g., a transgenic goat or a transgenic cow.

In a preferred embodiment, the EPOa-hSA fusion protein is secreted into a bodily fluid and the method further includes purifying the EPOa-hSA fusion protein from the bodily fluid.

In a preferred embodiment, the transgenically produced EPOa-hSA fusion protein is made in a mammary gland of a transgenic mammal, preferably under the control of a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific

promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is made in a mammary gland of the transgenic mammal, e.g., a ruminant, e.g., a dairy animal, e.g., a goat or cow.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk of a transgenic mammal at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In preferred embodiments the method further includes recovering EPOa-hSA fusion protein from the organism or from a product produced by the organism, e.g., milk, seeds, hair, blood, eggs, or urine.

In yet another embodiment, the EPOa-hSA fusion protein is produced in a transgenic plant.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3),and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method of making a transgenic EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion described herein. The method includes providing a transgenic animal, e.g., goat or a cow, which includes a transgene which provides for the expression of the EPOa-hSA fusion protein; allowing the transgene to be

expressed; and, preferably, recovering EPOa-hSA fusion protein, from the milk of the transgenic animal.

In preferred embodiments, the EPOa-hSA fusion protein is made in a mammary gland of the transgenic mammal, e.g., a ruminant, e.g., a goat or a cow.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk of the transgenic mammal, e.g., a ruminant, e.g., a dairy animal, e.g., a goat or a cow.

In preferred embodiments, the EPOa-hSA fusion protein is made under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk of a transgenic mammal at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method for providing a transgenic preparation which includes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein

described herein, in the milk of a transgenic mammal. The method includes: providing a transgenic mammal having an EPOa-hSA fusion protein protein-coding sequence operatively linked to a promoter sequence that results in the expression of the protein-coding sequence in mammary gland epithelial cells, allowing the fusion protein to be expressed, and obtaining milk from the mammal, thereby providing the transgenic preparation.

In a preferred embodiment, the EPOa-hSA fusion protein-coding sequence operatively linked to a promoter sequence is introduced into the germline of the transgenic mammal.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method for providing a transgenic preparation which includes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein, in the milk of a transgenic goat or transgenic cow. The method includes providing a transgenic goat or cow having an EPOa-hSA fusion protein-coding sequence operatively linked to a promoter sequence that results in the expression of the protein-coding sequence in mammary gland epithelial cells, allowing the fusion protein to be

expressed, and obtaining milk from the goat or cow, thereby providing the transgenic preparation.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a transgenic organism, which includes a transgene which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein.

In a preferred embodiment, the transgenic organism is a transgenic plant or animal. Preferred transgenic animals include: mammals; birds; reptiles; marsupials; and amphibians. Suitable mammals include: ruminants; ungulates; domesticated mammals; and dairy animals. Particularly preferred animals include: mice, goats, sheep, camels, rabbits, cows, pigs, horses, oxen, and llamas. Suitable birds include chickens, geese, and turkeys. Where the transgenic protein is secreted into the milk of a transgenic animal, the animal should be able to produce at least 1, and more preferably at least 10, or 100, liters of milk per year.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin

promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a transgenic cow, goat or sheep, which includes a transgene which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a herd of transgenic animals having at least one female and one male transgenic animal, wherein each animal includes an EPOa-hSA fusion protein transgene, e.g., a transgene which encodes an EPOa-hSA fusion protein described herein.

In a preferred embodiment, a transgenic animal of the herd is a mammal, bird, reptile, marsupial or amphibian. Suitable mammals include: ruminants; ungulates; domesticated mammals; and dairy animals. Particularly preferred animals include: mice, goats, sheep, camels, rabbits, cows, pigs, horses, oxen, and llamas. Suitable birds include chickens, geese, and turkeys. Where the transgenic protein is secreted into the milk of a transgenic animal, the animal should be able to produce at least 1, and more preferably at least 10, or 100, liters of milk per year.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein

or casein promoter. The milk specific promoter can is a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a pharmaceutical composition having a therapeutically effective amount of an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein, and a pharmaceutically acceptable carrier.

In a preferred embodiment, the composition includes milk.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_{\underline{4}}$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a kit having an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein, packaged with instructions for treating a subject in need of erythropoietin.

In a preferred embodiment, the subject is a patient suffering from anemia associated with renal failure, chronic disease, HIV infection, blood loss or cancer.

In another preferred embodiment, the subject is a preoperative patient.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a purified preparation of an EPOa-hSA fusion protein, e.g., an EPO-hSA fusion protein described herein.

In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 micrograms of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 milligrams of EPOa-hSA fusion protein.

In another aspect, the invention features, an EPOa-hSA fusion protein, or a purified preparation thereof, wherein the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 micrograms of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 milligrams of EPOa-hSA fusion protein.

In another aspect, the invention features, an EPOa-hSA fusion protein, or a purified preparation thereof, which includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3),and human serum albumin.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 micrograms of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 milligrams of EPOa-hSA fusion protein.

In another aspect, the invention features, an EPOa-hSA fusion protein, or a purified preparation thereof, which includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In preferred embodiments, the preparation includes at least 1, 10, or 100 milligrams of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, or 100 grams of EPOa-hSA fusion protein.

In another aspect, the invention features, a method of treating a subject, e.g., a human, in need of erythropoietin. The method includes administering a therapeutically effective amount of an EPOa-hSA fusion protein, e.g., an EPO-hSA fusion protein described herein, to the subject.

In a preferred embodiment, the subject is a patient suffering from anemia associated with renal failure, chronic disease, HIV infection, blood loss or cancer.

In another preferred embodiment, the subject is a preoperative patient.

In preferred embodiments the EPOa-hSA is administered repeatedly, e.g., at least two, three, five, or 10 times.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method of treating a subject in need of erythropoietin. The method includes delivering or providing a nucleic acid encoding an EPOa-hSA fusion protein, e.g., a fusion protein described herein, to the subject.

In a preferred embodiment, the nucleic acid is delivered to a target cell of the subject.

In a preferred embodiment, the nucleic acid is delivered or provided in a biologically effective carrier, e.g., an expression vector.

In a preferred embodiment, the nucleic acid is delivered or provided in a cell, e.g., an autologous, allogeneic, or xenogeneic cell.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[3]4-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method of making a transgenic organism which has an EPOa-hSA transgene. The method includes providing or forming in a cell of an organism, an EPOa-hSA transgene, e.g., a transgene which encodes an EPOa-hSA fusion protein described herein; and allowing the cell, or a descendent of the cell, to give rise to a transgenic organism.

In a preferred embodiment, the transgenic organism is a transgenic plant or animal. Preferred transgenic animals include: mammals; birds; reptiles; marsupials; and

amphibians. Suitable mammals include: ruminants; ungulates; domesticated mammals; and dairy animals. Particularly preferred animals include: mice, goats, sheep, camels, rabbits, cows, pigs, horses, oxen, and llamas. Suitable birds include chickens, geese, and turkeys. Where the transgenic protein is secreted into the milk of a transgenic animal, the animal should be able to produce at least 1, and more preferably at least 10, or 100, liters of milk per year.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the organism is a mammal, and the EPOa-hSA fusion protein is secreted into the milk of the transgenic animal at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-Gly-Gly-Gly)$_3$-Ser-Pro), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[$_3$]$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[$_3$]$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[$_3$]$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

advantageously from about 10 to about 20 amino acids. Amino acid sequences useful as linkers of EPOa and hSA include, but are not limited to, $(SerGly_4)_y$ (SEQ ID 1) wherein y is greater than or equal to 8, or $Gly_4SerGly_5Ser$ (SEQ ID 2). A preferred linker sequence has the formula $(SerGly_4)_4$ (SEQ ID 1). Another preferred linker has the sequence ((Ser-Ser-Ser-Ser-Gly)$_3$-Ser-Pro) (SEQ ID 4).

The EPOa and hSA proteins can be directly fused without a linker sequence. Linker sequences are unnecessary where the proteins being fused have non-essential N-or C-terminal amino acid regions which can be used to separate the functional domains and prevent steric interference. In preferred embodiments, the C-terminus of EPOa can be directly fused to the N-terminus of hSA or the C-terminus of hSA can be directly fused to the N-terminus of EPOa.

Recombinant Production

An EPOa-hSA fusion protein can be prepared with standard recombinant DNA techniques using a nucleic acid molecule encoding the fusion protein. A nucleotide sequence encoding a fusion protein can be synthesized by standard DNA synthesis methods.

A nucleic acid encoding a fusion protein can be introduced into a host cell, e.g., a cell of a primary or immortalized cell line. The recombinant cells can be used to produce the fusion protein. A nucleic acid encoding a fusion protein can be introduced into a host cell, e.g., by homologous recombination. In most cases, a nucleic acid encoding the EPOa-hSA fusion protein is incorporated into a recombinant expression vector.

The nucleotide sequence encoding a fusion protein can be operatively linked to one or more regulatory sequences, selected on the basis of the host cells to be used for expression. The term "operably linked" means that the sequences encoding the fusion protein compound are linked to the regulatory sequence(s) in a manner that allows for expression of the fusion protein. The term "regulatory sequence" refers to promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990), the content of which are incorporated herein by reference. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells, those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences) and those that direct expression in a regulatable manner (*e.g.*, only in the presence of an inducing agent). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to